

Measurements: Range of diameter of 30 specimens from the Philippines, Okinawa, and Palau, 0.87–1.34 mm (mean 1.06, SD 0.11); range of height (30 specimens), 0.64–0.94 mm (mean 0.79, SD 0.08); range of height/diameter ratio (30 specimens), 0.60–0.87 (mean 0.75, SD 0.06); range of protoconch diameter of 33 specimens from Philippines, Okinawa and Palau, 142 μ m–176 μ m (mean 157, SD 0.8).

Remarks: In teleoconch shape and surface sculpture this species resembles the species of the genus *Laroechea*. In addition to the lack of internal inner lip septum, *T. ohashii* differs from *Laroechea miranda* Finlay, 1927, *Laroechea secunda* Powell, 1937, and *Laroechea scitula* Marshall, 1993, in having a wide umbilicus and a small smooth protoconch (Figures 1A–C, 2A, B). *T. ohashii* is easily distinguished from *Laroecheopsis amplexa* Marshall, 1993, primarily by the reticulate surface sculpture and presence of a wide umbilicus. The new species is distinguished from *T. marshalli* primarily in having a broadly open umbilicus and coarser ornamentation. Both species have a similar meshlike ornamentation over the shell surface, but the intersections of the axial and spiral cords are spiny in *T. ohashii*, whereas they are not in *T. marshalli*.

Etymology: The species is named after Mr. Shu-ichi Ohashi, a professional SCUBA diver from Naha, Okinawa, Japan, who helped the authors in collecting the material.

Troглоconcha tessellata Kase & Kano, sp. nov.

(Figures 1D–F, 2C, D)

Type specimens: Holotype, NSMT Mo72830, 1.02 mm high, 1.11 mm wide; 7 paratypes, NSMT Mo72831.

Type locality: North of Kohama Island, Yaeyama Group, Okinawa (24°21.5'N, 123°58.9'E); depth 15 to 20 m; crevices; coral sand.

Distribution: Okinawa, Japan; only known from the type locality.

Description: Shell minute in size, naticiform, 1.11 mm in width in largest specimen, anomphalous, thin, fragile, opaque white in shell color, with width slightly greater than height. Spire elevated very slightly from body whorl. Periostracum unknown. Protoconch almost planispiral, smooth except for indistinct granules, about 0.16 mm in diameter, 1.25 in volution, tip narrowly rounded, and separated from teleoconch by slightly flared rim. Teleoconch just two volutions in largest specimen, rapidly expanded, evenly rounded, and separated by weakly impressed suture; end of mature body whorl descending obliquely and steeply. Sculpture of first half of teleoconch whorl prosocline, roundly curved, regularly spaced, sharp, 25 colabral axial riblets; after ½ volution teleoconch whorl

starting to bear spiral cords finer than axial riblets, first on middle of upper surface and adding successively on both sides of first one, finely granulated at intersections of axial riblets. Body whorl round-sided, sculptured with dense and very fine, prosocline axial and spiral cords. Aperture large, subcircular, moderately prosocline and interrupted at base of previous whorl. Outer lip thin and sharp, parietal area weakly convex, and inner lip slightly reflected at abaxial end. Soft part unknown.

Measurements: Range of shell diameter of eight specimens, including holotype, 0.75–1.14 mm (mean 0.91, SD 0.11); height, 0.69–1.02 mm (mean 0.83, SD 0.10); height/diameter ratio, 0.90–0.96 (mean 0.91, SD 0.02).

Remarks: This species is represented only by eight empty shells and is known only from the type locality, where it is associated with *T. ohashii*. It has a smooth protoconch, and sculpture pattern in the early teleoconch whorl and apertural features almost identical to *T. ohashii*. *T. tessellata* has a similar shell form and protoconch morphology to those of *Laroecheopsis amplexa*, but the meshlike sculpture pattern over the teleoconch surface of the new species differs from the shallow pits in the early whorls and spiral threads in the later whorls of the latter species. The allocation of this species to *Troглоconcha* gen. nov. is based on the greater similarity of the shell to *T. ohashii* rather than to *L. amplexa*.

Troглоconcha tessellata most closely resembles *Troглоconcha marshalli* from the upper Oligocene of France in its overall shell characters, strongly suggesting congeners for both species. The new species is only separable from *T. marshalli* in the absence of an umbilicus. It is also distinguishable from *T. ohashii* in its higher proportion of shell form, less steeply inclined outer lip, much finer meshlike ornamentation, and lack of open umbilicus.

Etymology: From the Latin, *tesselatus* (adv.), meaning tessellated with reference to the finely reticulate ornamentation of the whorls.

Acknowledgments. Many people helped us in collecting the materials: I. Hayami (Kanagawa University), S. Ohashi, S. Kinjo, H. Kinjo, M. Uchima, K. Ogura, M. Taniguchi (Okinawa), J. Cabrera (National Museum, Manila), R. Gibson (Vanuatu), G. Paulay, L. Kirkendale, C. Meyer (University of Florida), Y. Yamazaki (Palau), B. Richer de Forges and the captain and crew of R/V *Dawa*, ORSTOM (New Caledonia), M. Cathrein (Christmas Island), D. Gerhard (Cocos Keeling), A. Jimwereiy (Nauru), T. Furuta (Pohnpei), and M. Yasui (Yap). We thank T. Isamu (Division of Marine Resources, Palau), J. Starmer (Coral Reef Research Foundation, Palau), S. Slack-Smith (Western Australian Museum), and D. Slip (Parks Australia, Christmas Island) for their assistance in obtaining research permits. We also thank C. Mayer for his editorial assistance and valuable suggestions. T.K. received financial support from grants from the Ministry of Education, Science and Culture, Japan (nos. 06454003, 08041162, 11691196 and 11833018), the Fujiwara Natural History Foundation, and the Research Institute of Marine Invertebrates.

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Reproductive Cycle of the Bivalves *Ensis macha* (Molina, 1782) (Solenidae), *Tagelus dombeii* (Lamarck, 1818) (Solecurtidae), and *Mulinia edulis* (King, 1831) (Mactridae) in Southern Chile

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Abstract. The reproductive cycles of the bivalves *Ensis macha* (Molina, 1782), *Tagelus dombeii* (Lamarck, 1818), and *Mulinia edulis* (King, 1831) were studied at six sites in southern Chile (38–43°S) from November 1996 to December 1997. Samples of *E. macha* came from three subtidal shallow depths; those of *T. dombeii* from two subtidal depths and one intertidal site; and samples of *M. edulis* originated in one subtidal shallow depth and one intertidal site. Thirty specimens were collected monthly for standard histological analyses. Water samples were also collected to determine salinity, temperature, and chlorophyll *a* content. In general, the reproductive cycles of the three species were characterized by long spawning periods, beginning during late spring-summer. In some cases, that period extended during autumn-winter until the following spring. The gonads of most of the populations showed quite short recovery periods, with the exception of populations located farther south, which needed more time to begin a new cycle. Comparison of subtidal versus intertidal populations showed that the gonad stages developed more slowly for the latter populations. The earlier results show that variability exists in the timing of gametogenic cycles of *E. macha*, *T. dombeii*, and *M. edulis* along the coast of southern Chile. No significant relationship was found between seasonal variability of reproductive stages and seasonal variability of water characteristics. Among these characteristics, water temperature and chlorophyll *a* content were the most important. Potential fecundity varied geographically in *E. macha* and *T. dombeii*, whereas, in general, no variability was observed in mean sizes of oocytes of the three species. These results must be taken into account when management plans are designed; thus, the timing of the gametogenic cycles of bivalves of economic importance must be studied along their full geographic ranges.

INTRODUCTION

Knowledge of the reproductive cycles of marine invertebrates of economic importance is basic to culture activities and management of natural stocks. In that way, it is possible to regulate fishery activities and set up closing seasons to preserve the species, via the protection of reproductive individuals (e.g., Defeo 1987, 1989, 1993).

The reproductive cycles of different bivalve species are unique for each population, varying according to geographic location (Sastry, 1979). The high seasonality of the environment in medium to high latitude locations results in annual cycles, with gametogenesis during winter, and spawning during the spring-summer season. In contrast, the reproductive cycle in low latitude locations is characterized by long or continuous spawning periods (Heffernan & Walker, 1989; Heffernan et al., 1989a, b). Environmental factors such as water temperature and salinity, photoperiod, and food resource availability have been mentioned as concrete causes for that latitudinal variability (Giese & Pierse, 1977; Mackie, 1984). The latitudinal variability in the reproductive cycle of bivalves

has been particularly studied in species of economic importance (Tarifeño, 1980; Manzi et al., 1985; Heffernan et al., 1989a, b; Laasuy & Simons, 1989; Kanti et al., 1993; Urban & Campos, 1994; Villalejo-Fuerte et al., 1996a; Gallardo & Weber, 1996).

Apart from the variability in reproductive cycles related to geographic variation, it has been shown that zonation across shore also influences some of the characteristics of the reproductive cycles such as the production of somatic and sexual tissue and differences in fecundity and size of oocytes (McLachlan, 1974; Griffiths, 1981; Harvey & Vincent, 1989, 1991; Richardson, 1993; Walker & Heffernan, 1994; Brousseau, 1995). For example, Borrero (1987) found three important differences between subtidal and intertidal populations of the mytilid *Geukensia demissa* (Dillwyn) in South Carolina, USA: time of the onset of gametogenesis, time of occurrence of spawning, and length of time remaining in a mature reproductive condition before spawning. That was probably due to changing conditions in submergence throughout the tidal cycle, which result in changing environmental tem-

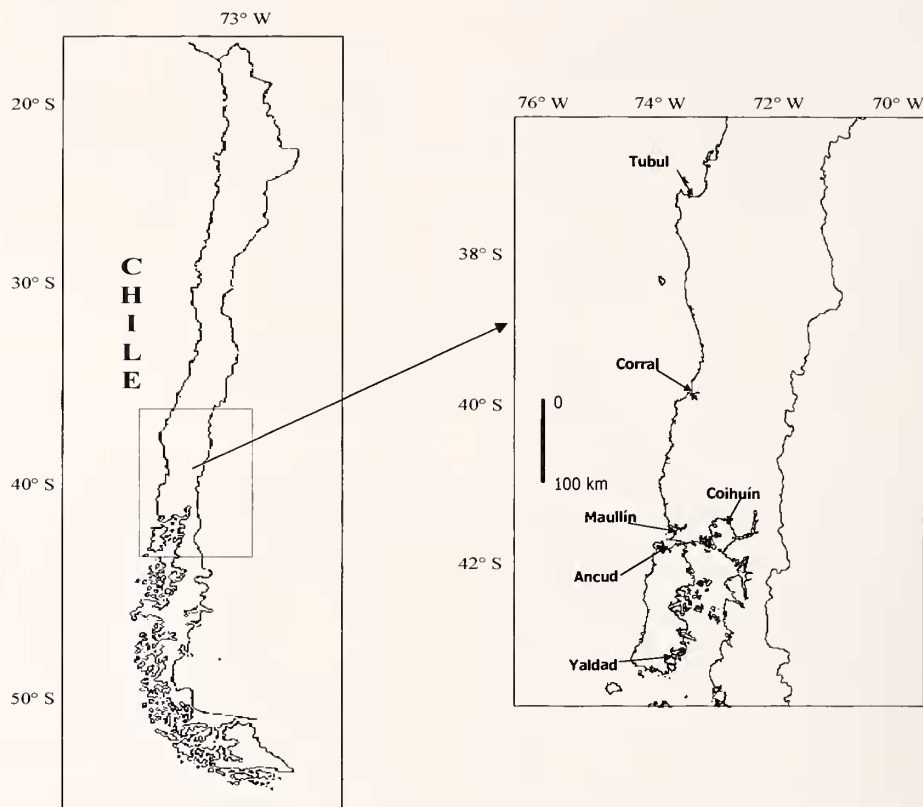


Figure 1. Location of sampling sites at the coast of south central Chile.

peratures and time for feeding, conditions that may influence the reproductive output of invertebrates (Barber & Blake, 1981; Bayne & Newell, 1983).

The coast of southern Chile (38–43°S) is characterized by small bays and numerous microtidal estuaries. Some of the most common bivalves are *Ensis macha* (Molina, 1782) (Solenidae); *Tagelus dombeii* (Lamarck, 1818) (Solecurtidae); and *Mulinia edulis* (King, 1831) (Mactridae). *Ensis macha*, *Tagelus dombeii*, and *Mulinia edulis* occur along a wide latitudinal range of the Chilean coast, the first species from Caldera (approx. 27°S) to Magallanes (55°S), and the latter two species throughout all the Chilean coast (Osorio et al., 1979). They are among the most common bivalves subjected to commercial fisheries along the coast of southern Chile (approx. 38–43°S). Approximate commercial sizes are 100–180 mm for *E. macha*, 70–90 mm for *T. dombeii*, and 50–70 mm for *M. edulis*. Landing fisheries along the Chilean coast started in 1988 for *E. macha*, 1965 for *T. dombeii*, and 1994 for *M. edulis*.

In recent years, landing figures for *E. macha*, *T. dombeii*, and *M. edulis* in southern Chile (about 38–43°S, Arauco to Quellón, Figure 1) represented an average of 99.7, 99.5, and 80.7% of the total national landing figures (data from 1988–1997 for *E. macha* and *T. dombeii*, and from 1994 to 1997 for *M. edulis*). During the period

1988–1997, the highest landing figures from *E. macha* and *T. dombeii* were 8595 (1991) and 7260 annual tons (1988), respectively. From 1994–1997, the maximum landing for *M. edulis* reached 2553 annual tons (1994). The vast majority (approx. 90%) of landings are used in the canning industry (Sernapesca, 1998).

Despite the economic importance of these three species, there are few studies which deal with the effect of geographic variability on their reproductive biology. Santos-Salas et al. (1998) and Aracena et al. (1998) described growth and feeding of juveniles and the reproductive cycle of *E. macha* in shallow waters of Golfo de Arauco (approx. 38°S). For *M. edulis* there is only one study on the external morphology of larvae (Fuentes, 1988), and another study on production of spats (Paredes & Hernández, 1986). For *T. dombeii* there are several studies on its reproductive cycle at several localities on the Chilean coast, showing variations in its reproductive cycle (Lasen, 1979; Fierro, 1981; Arratia, 1998).

Because of the variability of coastal waters along the Chilean coast (Brattström & Johanssen, 1983; Strub et al., 1998; Viviani, 1979), it is reasonable to expect some variability in reproductive cycles along the latitudinal ranges of these species. The purpose of the present study was to analyze the reproductive biology of *E. macha*, *T. dombeii*, and *M. edulis*, (Figure 2) at different areas of a coastal

range spanning approximately 600 km of the southern Chilean coast. Because *E. macha* lives primarily under water, we obtained only subtidal samples for this species. For *T. dombeii* and *M. edulis*, however, we obtained intertidal and subtidal samples. Thus, for *T. dombeii* and *M. edulis* we were able to analyze populations living at different latitudes and depths.

MATERIALS AND METHODS

Study Area

Samples were collected from six sites on the coast of southern Chile (Figure 1). Subtidal samples of *E. macha* were collected from Tubul, Golfo de Arauco (37°14'S, 73°29'W), Bahía de Corral (Corral hereafter) (39°50'S, 73°28'W), and Bahía de Ancud (Ancud hereafter) (41°50'S, 73°47'W). Subtidal samples of *T. dombeii* came from Tubul and Corral; intertidal samples of this species were collected from Coihufín, Golfo del Reloncaví (41°28'S, 72°41'W). Subtidal samples of *M. edulis* were collected at Maullín (41°39'S, 73°37'W); intertidal samples of this species came from Yaldad (43°07'S, 73°44'W) (Figure 1).

Sampling and Treatment of Samples

Samples were collected from November 1996 to December 1997. Subtidal samples were collected by semi-autonomous diving from shallow water beds (7–14 m depth). Intertidal samples were collected during spring low tides. Due to rough sea conditions, no samples were collected during April in Corral and during May at Tubul and Corral.

For histological analysis of the gonads, samples of 30 specimens were collected from each one of the study sites. The bivalves were kept at low temperature (3°C) to be processed within 24 hours after collection. After dissection, the gonads were fixed in aqueous Bouin's fixative. After embedding in paraffin, 7 µm serial sections were cut and stained with hematoxylin and eosin (Bancroft & Stevens, 1977). Ten sections of the gonad of each specimen were examined under the light microscope to determine the gonadal organization and the seasonal gametogenic cycle. The following categories of gonad development were used in this study (cf. Peredo et al., 1987; Brousseau, 1995).

Early Active. Phase of gamete proliferation and development. Gonadal follicles are small and have thick walls; the interstitial tissue is abundant and disseminated among the gonadal follicles. In males, spermatogonia are close to the follicular walls, while few spermatids and spermatocytes are located near the center of the follicles. In females, oogonia can be seen embedded in the follicular walls; pre-vitellogenic oocytes and vitellogenic oocytes with cytoplasm extend into the lumen of the follicles.

Late Active. This is the phase of gamete maturation. In

both sexes a reduction of gonias and an increase in the mature gametes (oocytes and spermatozoa) can be seen. In males the sperm form radially oriented columns with their tails toward the center of the follicles. In the female gonad, vitellogenic oocytes are more numerous, and some mature oocytes are free in the lumen of the follicles.

Ripe. Gonadal follicles are expanded in these stage with their walls being very thin and with a lower number of early stage cells. In males, mature sperm form dense masses and cells. In females, the follicles are crowded together and filled with mature oocytes.

Partially Spawning. In both sexes the follicles still contain gametes, but these are less numerous than in the ripe stage. It is still possible to see gametes in early stage (spermatids and vitellogenic oocytes attached to the wall).

Spent. In both sexes, most of the follicles are devoid of gametes with some residual mature spermatozoa or oocytes.

Recovery. Most of the follicles are devoid of gametes, although some follicles have a few residual gametes. The interstitial tissue has increased and surrounded the follicles.

The stereometric technique of Weibel (1969) was used for fecundity determination, i.e., the volume of different cellular components was determined from the gonad analyses, through the relationship between the surface of that component and the total surface (Neuer, 1966). The diameter of 100 oocytes (from different females) was measured, using an eyepiece graticule calibrated with a stage micrometer. Measurements were made along the longest and the shortest axis of the oocytes. From these data, mean oocyte size and standard deviation were obtained. Maturity of the oocytes was determined according to Peredo et al. (1987) and Masello (1987), i.e., free oocyte in the light of the follicle with cytoplasm of the lumpy aspect and acidophile; large rounded nucleus; clearer color than cytoplasm with a very clear nuclear membrane and granulated chromatin; large nucleoli in the interior to which small ones can be added.

Water samples from the subtidal sampling sites (Tubul, Corral, Maullín, and Ancud) were collected from about 50 cm above the bottom to determine temperature, salinity, and chlorophyll *a* content. At Coihufín and Yaldad (intertidal sites) water samples were collected during rising tides (about 50 cm depth). Temperatures were measured *in situ* with a mercury thermometer ($\pm 0.1^\circ\text{C}$). Salinity was measured with a portable salinometer Hydro-bios. The chlorophyll *a* content was measured after the filtration of 2 liters of water in Milipore filters with 0.45 µm of opening. The filters were kept at low temperatures (-7°C) and in darkness. After a short period (5–7 days), they were kept in 90% acetone for 24 hours to extract pigments, and centrifuged at 3500 rpm for 15 min. The absorbance of the supernatant was measured at 750 and 665 nm (Strickland & Parsons, 1972).

One-way analysis of variance (Sokal & Rohlf, 1995)

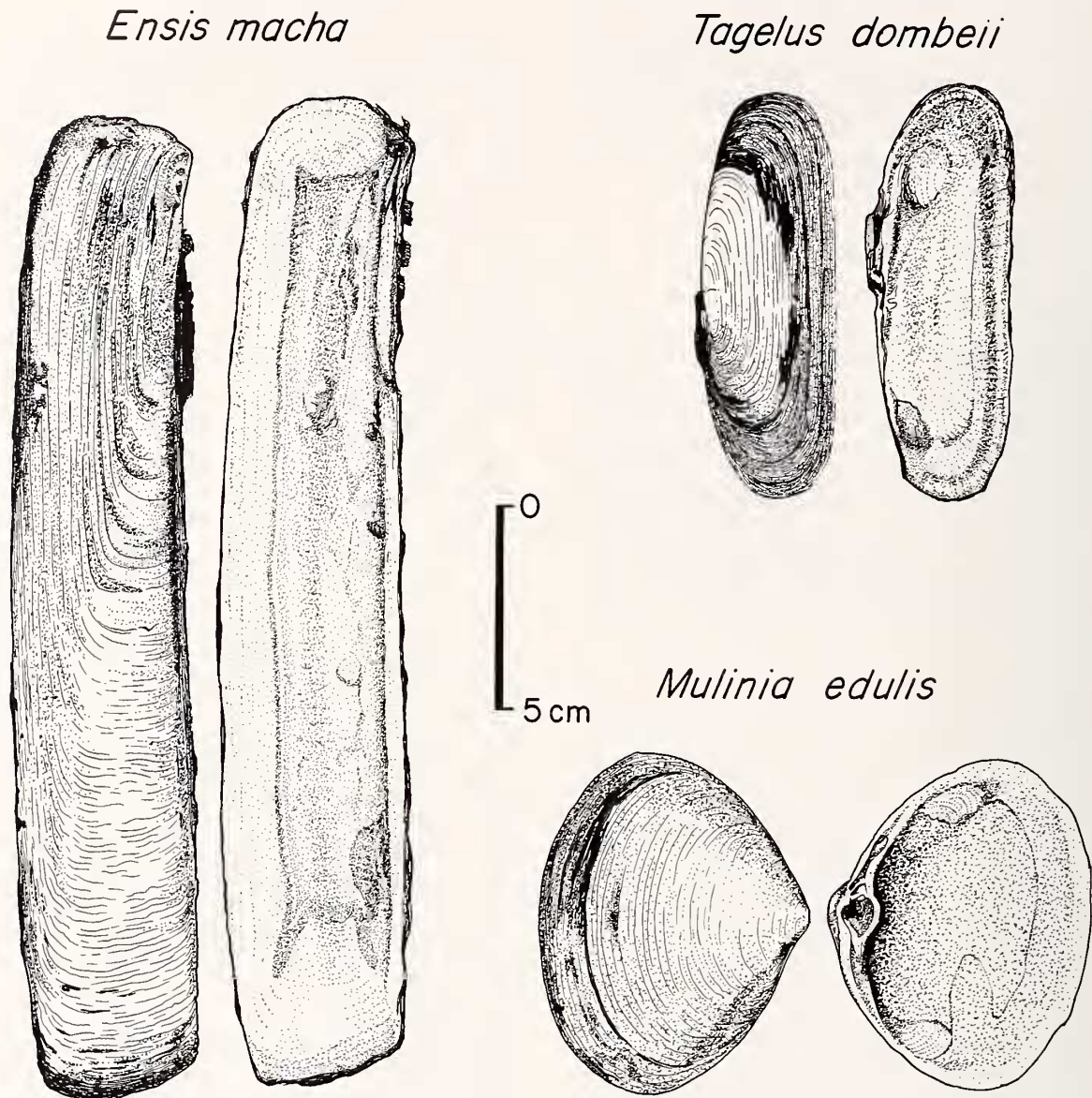


Figure 2. External and internal views of shells of *Ensis macha*, *Tagelus dombeii*, and *Mulinia edulis*.

was used to compare the mean potential fecundity among bivalves of the same species collected at different sites. If the analysis of variance indicated significant differences among means ($P < 0.05$), these were compared using the *a posteriori* Tukey's multiple comparison test (Day & Quinn, 1989). Due to the fact that mean oocyte diameter did not have a normal distribution, a non-parametric analysis (Kruskal-Wallis ANOVA) was used. Simple regression analysis (Sokal & Rohlf, 1995) was carried out to evaluate relationships between percentages of mature (ripe stage) and spawned (partially spawned and spent stage) and variability of water temperature and chlorophyll *a* water content.

RESULTS

Water Characteristics

Figure 3 shows the temporal variability in temperature, salinity, and chlorophyll *a* content of water at each study site. Water temperature showed small variability at Tubul: from 12°C in March to 14.5°C in April and July. More seasonal variability was found farther south; from a minimum of 11, 10, 10, 12 and 10°C during winter time to a maximum of 13.6, 16, 18.5, 16, and 15°C during late spring-summer at the waters of Corral, Maullín, Coihufn, Ancud, and Yaldad, respectively (Figure 3). Water salinity varied little at the shallow waters of the bays of Tubul.

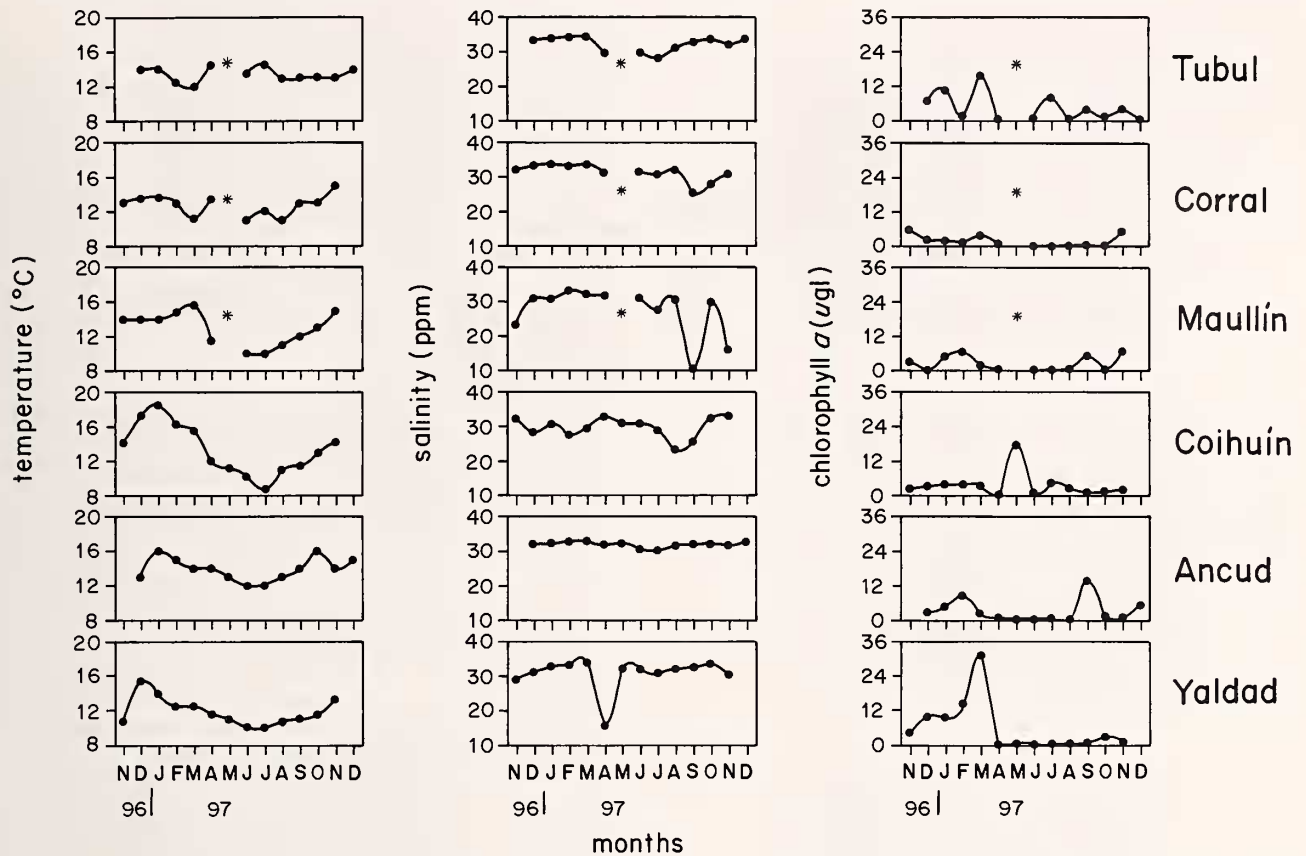


Figure 3. Temporal variability of temperature, salinity, and chlorophyll *a* content in the waters of the sampling sites. * = no data

Corral, and Ancud (from about 28–34 ppm). More variability was found at the shallow waters of Maullín (10–33 ppm), and at the intertidal sites of Coihuin and Yaldad (15–34 ppm). In general, the highest content of chlorophyll *a* at all study sites was found during summer (December–February) and spring months (September–November), whereas the lowest occurred during late fall and winter. During late summer of 1997 (March), the intertidal waters of Yaldad registered the highest chlorophyll *a* value found in this study (31.2 µg/l) (Figure 3).

Morphology of Gonads

Microscopic analyses revealed that *E. macha*, *T. dombeii*, and *M. edulis* are dioecious species without external sexual dimorphism. While no color differences exist in the gonads of *E. macha* and *T. dombeii*, the gonads of *M. edulis* vary in color, being dark royal purple in females, and orange in males. The gonad structure is similar in the three species; it is embedded in the visceral mass together with the hepatopancreas and gut. It does not have any kind of enveloping sheet; in some regions it is dissected by muscular strings originating in the body wall.

Gametogenic Cycles

Ensis macha. Figure 4 shows the frequency of the different gonad stages of *E. macha* at the shallow waters of Tubul, Corral, and Ancud. At the beginning of the study (November–December 1996), the three populations were in late active and ripe stages. During January, 23% and 13% of the individuals collected at Corral and Ancud had partially spawned. Twenty-seven percent of the animals collected during February in Tubul had reached this stage. During late summer (March), 100%, 93%, and 50% of the populations of Tubul, Corral, and Ancud, respectively, were partially spawned. One hundred percent of the animals of Tubul and Ancud were in this stage during April. During the winter and spring months, the three populations showed significant differences in their gametogenic cycles. During June and July, about 55% of the specimens of *E. macha* in Tubul had their gonads in early and late active stages. From June to August, percentages of ripe individuals varied (approx. 20–50%). During August, partially spawned individuals were collected again (approx. 40%); this last stage persisted during the rest of the spring (September–December). During June–August,

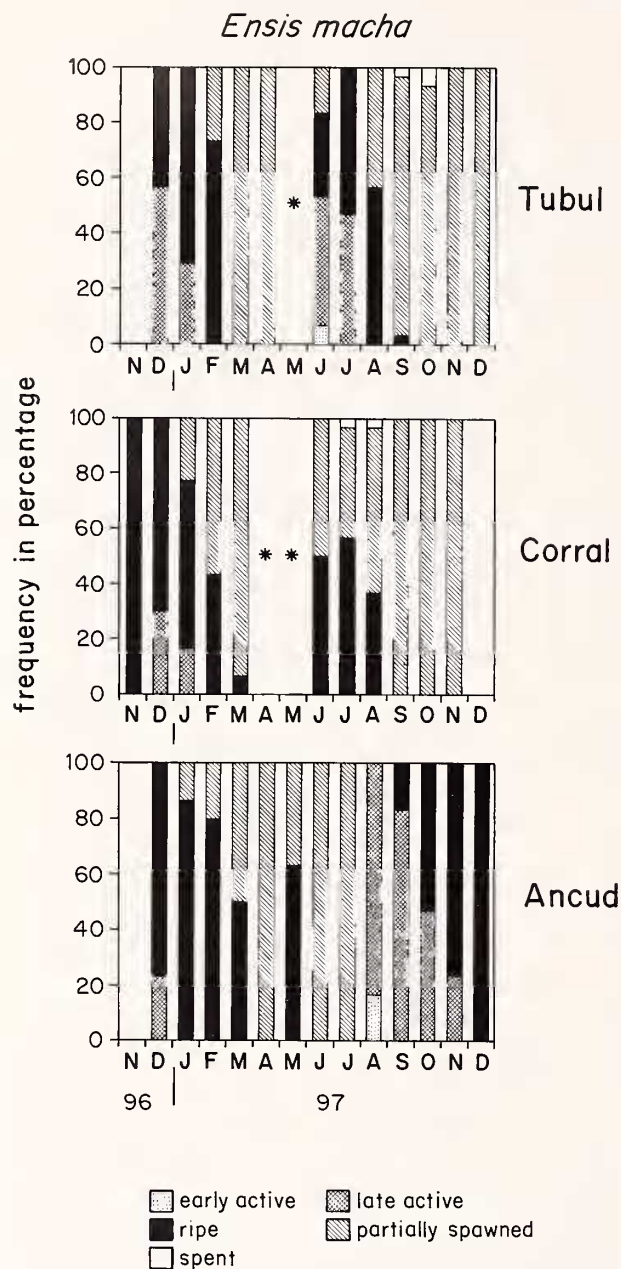


Figure 4. Seasonal variability in the frequencies (percentages) of the different stages of the gametogenic cycle of the gonad of *Ensis macha* at the shallow subtidal of Tubul, Corral, and Ancud. * = no data

the population of Corral was represented by ripe and spawned animals in similar proportions. From September to November, 100% of the animals were in partially spawned stage. The specimens collected during May in Ancud were ripe and partially spawned; during June–July, 100% of them were in this last stage. From August to November, individuals were either recovering (late active

stage) or mature (ripe stage). During December, 100% of the animals collected in Ancud were mature.

Tagelus dombeii. Figure 5 shows the sequence of the reproductive cycle of *T. dombeii* at the shallow waters of Tubul and Corral and at the intertidal of Coihuin. During the first 2 months of the study (November and December 1996), the three populations were in different reproductive stages. Whereas the animals of Tubul were in late active or ripe stage, those of Corral and Coihuin were ripe or spawned (primarily at Corral, 97% during December). During January, the individuals of Tubul had yet not spawned, whereas most of the individuals from Corral and Coihuin were spawning, with some specimens also in the ripe stage (5% and 20%, respectively). The beginning of a new cycle (individuals in early active stage) was observed in June for the individuals of Tubul and Coihuin, and in July for the specimens of Corral. During late winter (August–September), the population of Tubul was spawning, even when ripe animals were also collected. At the same time, the populations of Corral and Coihuin were still in early and late active stages. During the spring (October–December), most of the specimens collected at Tubul were spawning. All the individuals collected at Corral were in late active stage during October, and ripe during November. In Coihuin, most of the individuals (approx. 95%) were ripe in October; and in November, half of the population was partially spawned.

Mulinia edulis. The reproductive cycle of *M. edulis* at the shallow waters of Maullín and at the intertidal of Yaldad is shown in Figure 6. During November 1996, different reproductive stages were found (early active, late active, and ripe stage) at both sites. During summer (December–March), both populations were in ripe and partially spawned stages, with the highest percentage of spawned individuals found at the end of the summer (approx. 50% in Maullín and 77% in Yaldad). Both populations were in similar stages during April to June. Specimens collected at Maullín during July were in recovery, ripe, and spawn stages, quite a similar situation (but without recovery stages) to that observed for the intertidal population of Coihuin. During August, both populations were in similar stages of the gametogenic cycle. From September to November, the specimens of Maullín were spawning, while those of Yaldad had begun a new cycle (early and late active stages), an assertion supported by the dominance of ripe stages during November.

Gametogenic Cycles and Water Characteristics

Table 1 shows the results of regression analyses carried out between the seasonal variability in the percentages of mature (ripe stages) and spawned individuals (partially spent and spawned) and the temporal variability of water characteristics. The temporal variability in the percentage of mature females of *E. macha* at Tubul was positively correlated with that of chlorophyll *a* content. The tem-

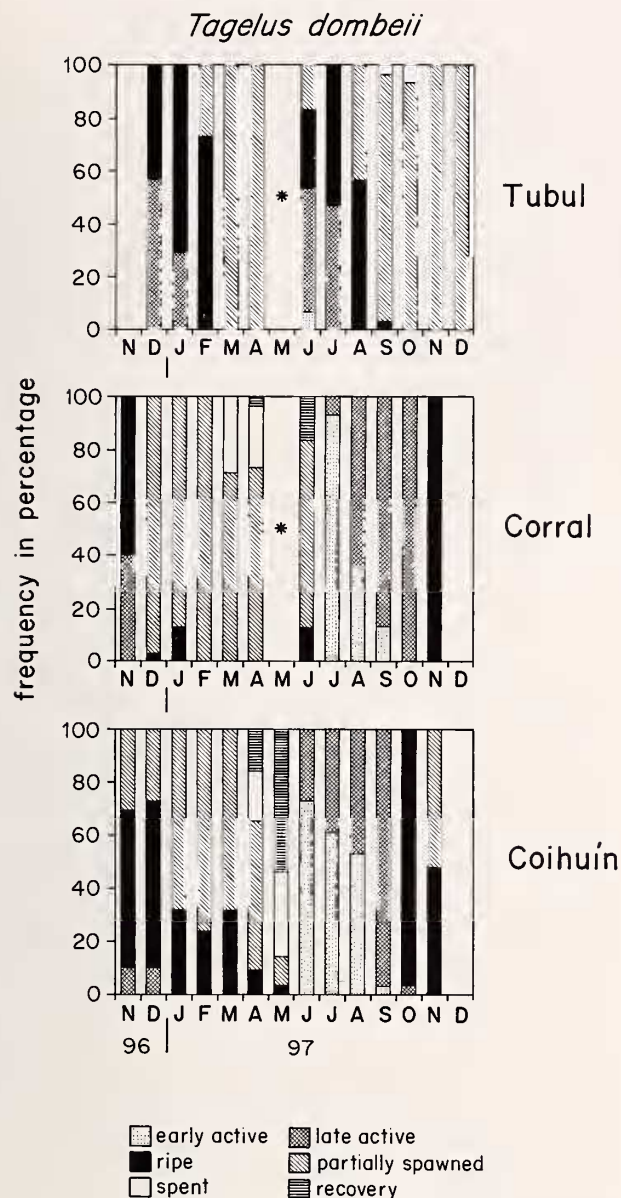


Figure 5. Seasonal variability in the frequencies (percentages) of the different stages of the gametogenic cycle of the gonad of *Tagelus dombeii* at the shallow subtidal of Tubul and Corral and the intertidal site of Coihuín. * = no data

poral variability in the percentages of spawned specimens (either the whole population or males and females by themselves) was also correlated with the chlorophyll *a* content, but inversely. Only the females of *E. macha* showed a significant relationship to the water characteristics at Corral; mature females were more abundant when chlorophyll *a* was higher, whereas spawned females peaked when chlorophyll *a* was lower. The results found from *E. macha* at Ancud showed that mature individuals were more abundant when temperatures were higher,

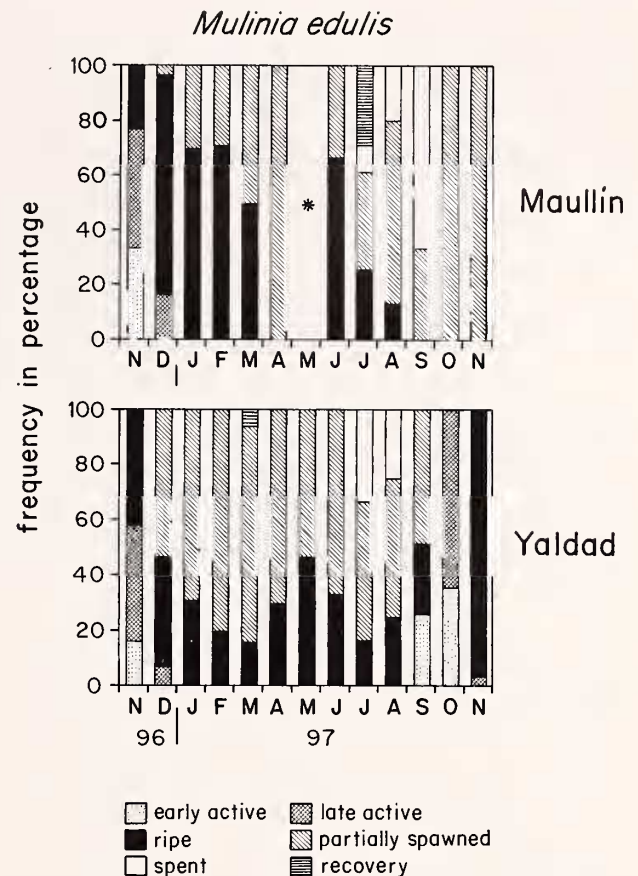


Figure 6. Seasonal variability in the frequencies (percentages) of the different stages of the gametogenic cycle of the gonad of *Mulina edulis* at the shallow subtidal of Maullín and the intertidal site of Yaldad. * = no data

whereas spawned females peaked when temperatures were lower. At the intertidal site of Coihuín, a significant correlation was found between the temporal variability of the reproductive stages of *T. dombeii* and physical factors; thus, percentages of total individual spent, mature females and spawned individuals were more abundant when water temperatures were higher. Similarly, percentage of mature females of *M. edulis* at the intertidal site of Yaldad peaked when water temperatures were higher (Table 1).

Diameter of Oocytes and Potential Fecundity

The mean diameter of oocytes was 50–51 μm for *E. macha*, 38–39 μm for *T. dombeii*, and 41–42 μm for *M. edulis*, without significant differences between sites ($P > 0.05$, Table 2). Mean potential fecundities were higher for *E. macha*, particularly in Tubul and Corral where those fecundities (approx. $18\text{--}19 \times 10^6$ oocytes per individual) were significantly higher ($p < 0.05$) than that estimated for the population sampled at Ancud (Table 2). The high-

Table 1

Results of the regression analyses carried out between the percentages of mature and spawned animals and the temporal variability of water temperature and chlorophyll *a* content at the study sites. Results are only given for those analyses which rendered significant correlations.

| Species | Study site | Regression equation | r | p |
|------------------------|------------|---|-------|------|
| <i>Ensis macha</i> | Tubul | % mature females = $-5.74 + 6.55 \text{ chlor } a$ | 0.88 | 0.00 |
| | | % spawned males and females = $83.28 - 5.65 \text{ chlor } a$ | -0.58 | 0.04 |
| | | % spawned females = $86.59 - 6.06 \text{ chlor } a$ | -0.59 | 0.04 |
| | | % spawned males = $724.96 - 49.79 \text{ chlor } a$ | -0.81 | 0.00 |
| | Corral | % mature females = $10.79 + 14.44 \text{ chlor } a$ | 0.69 | 0.02 |
| | | % spawned females = $89.27 - 16.04 \text{ chlor } a$ | -0.71 | 0.01 |
| | Ancud | % mature males and females = $-197.41 + 17.51^\circ\text{C}$ | 0.61 | 0.03 |
| | | % mature females = $-220.67 + 18.88^\circ\text{C}$ | 0.59 | 0.03 |
| <i>Tagelus dombeii</i> | Coihuín | % spawned females = $313.46 - 19.91^\circ\text{C}$ | -0.58 | 0.04 |
| | | % population spent = $-55.45 + 6.67^\circ\text{C}$ | 0.62 | 0.02 |
| | | % mature females = $-66.78 + 7.20^\circ\text{C}$ | 0.68 | 0.01 |
| | | % spawned females = $-62.53 + 6.66^\circ\text{C}$ | 0.68 | 0.01 |
| | | % spawned males = $-74.78 + 8.2^\circ\text{C}$ | 0.67 | 0.01 |
| <i>Mulinia edulis</i> | Yaldad | % mature females = $-103.68 + 10.12^\circ\text{C}$ | 0.57 | 0.04 |

est mean potential fecundity for *T. dombeii* was estimated for the subtidal population sampled at Tubul. That fecundity (approx. 11×10^6 oocytes per individual) was significantly higher than that estimated for the subtidal population of Corral (8×10^6 oocytes per individual) and the intertidal samples of Coihuín (approx. 6×10^6 oocytes per individual), the last ones without significant differences between them (Table 2). The estimated mean potential fecundities estimated for the subtidal and intertidal populations of *M. edulis* (Maullín versus Yaldad) ranged from approx. $10\text{--}14 \times 10^6$ oocytes per individual without significant differences between them (Table 2).

DISCUSSION

The results of this study show that in the study area *E. macha*, *T. dombeii*, and *M. edulis* have an annual cycle of reproduction with periods of extensive spawns beginning during late spring-summer (November 1996 to February 1997). That period extends until autumn, and in some cases continues without stopping until next spring as was observed clearly in the intertidal population of *M. edulis* in Yaldad. In general, the recovery periods of the gonads are quite short and extend for more than 2 months just in the populations of *T. dombeii* in Corral and Coihuín, being the population studied of this species at the last place with the clearest annual cycle (i.e., all the gonad stages well represented).

The reproductive cycle of *E. macha* in Tubul and Corral was rather similar, with a quick recovery of the gonad during winter, after which spawning extended until the end of the study. The population of Ancud showed a more marked annual cycle with a new maturation period during the spring continuing toward the summer: spawning began during early summer (January 1997).

The reproductive cycle of *T. dombeii* of Tubul differed from that of Corral and Coihuín. The individuals collected from the first site began a new cycle during winter (June-July) which resulted in ripe individuals and spawning throughout spring. On the other hand, the beginning of a new cycle for the populations of Corral and Coihuín spanned a longer period of time; consequently, no spawning was found for Corral at the end of this study, whereas about 50% of the individuals of Coihuín were in this stage for that time. Thus, the gonads of the populations of *T. dombeii* located farther south take a longer time to go through all the stages before spawning.

The gametogenic cycle of *M. edulis* also showed intersite variability. While the subtidal population showed a continuous spawning and no beginning of a new cycle during the study period, the intertidal populations (also spawning almost all year round) showed the beginning of a new reproductive cycle during early spring.

The earlier results show a variability in the gametogenic cycles of *E. macha*, *T. dombeii*, and *M. edulis* along the geographic range studied.

Sastry (1979) considered water temperature as one of the most important factors in the regulation of the different stages of the reproductive cycle in marine invertebrates. Indeed, temperature is one of the main causes of differences in the timing of gametogenesis and spawning of different populations of the same species (Ropes, 1968; Tarifeño, 1980; Manzi et al., 1985; Urban & Campos, 1994; García-Domínguez et al., 1996). Vilalejo-Fuerte et al. (1996a) showed that in Baja California, Mexico, the increase in temperature inhibits gametogenesis in the cockle *Laevicardium elatum* (Sowerby). In other species of bivalves located in the Gulf of California, the spawning period is directly related to decrease of water tem-

Table 2

Mean size of oocytes and mean potential fecundity (with standard deviations in parentheses) for each species at the study sites. A summary of the statistical analyses (F and p; see Material and Methods) is also given. The same capital letters indicate no significant differences in the fecundity comparisons; different letters for the opposite results (i.e., significant differences).

| Species | Study site | K-W ANOVA | | Mean potential fecundity (millions of oocytes per individual) | ANOVA | |
|------------------------|------------|-----------|------|---|-------|------|
| | | F | p | | F | p |
| <i>Ensis macha</i> | Tubul | 1.79 | 0.41 | 18,267.243 (4,369,424) | 6.26 | 0.00 |
| | Corral | | | 19,612.243 (1,573,002) | | |
| <i>Tagelus dombeii</i> | Ancud | 0.51 | 0.77 | 14,676.729 (3,105,274) | 11.98 | 0.00 |
| | Tubul | | | 11,329.204 (3,286,167) | | |
| | Corral | | | 8,116.200 (2,364,978) | | |
| | Coihuín | | | 5,778.686 (1,750,145) | | |
| <i>Mulinia edulis</i> | Maulín | 0.42 | 0.51 | 14,155.714 (5,547,323) | 3.79 | 0.07 |
| | Yaldad | | | 10,259.714 (3,031,790) | | |

perature (Villalejo-Fuerte et al., 1995). There are, however, other species in which the water temperature does not influence gametogenic cycles (García-Domínguez et al., 1996).

In this study, we found that water temperature affected in some way the timing of the gametogenic cycle of *E. macha* in Ancud, *T. dombeii* in Coihuín, and *M. edulis* in Yaldad. Ancud was the southern study site for *E. macha*, while Coihuín and Yaldad were the intertidal sites. It is indeed possible that at those places, a wider temperature variability occurred than that measured here, which may explain the above-mentioned relationship between timing of reproduction and water temperature.

Food resources are also important, and the percentage of individuals in different gonad stages is related to food amount and availability, primarily during the final period of maturation of the gametes (late active and ripe stages) and during the period of spawning (Sastry, 1979; Bayne & Newell, 1983; MacDonald & Thompson, 1985; Villalejo-Fuerte et al., 1996a). In this way, the females have enough energy to carry out the vitellogenic process, and later, larvae also have enough food for subsistence. The direct and significant relationship between the percentage of mature females of *E. macha* and *T. dombeii* and the concentration of chlorophyll *a* in the localities of Tubul and Corral suggest that food is the main cause of final maturation of the oocytes. The other populations could obtain that energy from that accumulated in their body or from another food type different to the phytoplankton (e.g., dissolved organic matter). For an intertidal population of *Semele solida* (Gray) in Coihuín, Arratia (1998) determined a continuous reproductive cycle, with liberation of gametes during the 15 months of the study, even during periods of low food concentration. Similar results were found by Jaramillo & Navarro (1995) for the mytilid *Anlaconmya ater* in Yaldad. It has been demonstrated or suggested for bivalves that the transfer of nutrients exists from the somatic tissue toward the gonad during gametogenesis (Sastry & Venn, 1979; Barber & Blake, 1981; Villalejo-Fuerte et al., 1996b). Le Pennec et al. (1991) demonstrated for the pectinid *Pecten maximus* L. that other pathways exist for energy incorporation to the development of gametes, i.e., the recycling of atresic material and direct transfer of metabolites from the intestinal loop to the developing gametes. Sastry (1979) also mentioned the importance of dissolved organic matter, bacteria, and organic aggregates as a food source for bivalves, apart from the usual phytoplankton. Clasing et al. (1998) found at the intertidal flats of Coihuín that *Semele solida* spawns during autumn-winter when concentrations of phytoplankton are low. These authors stated that because *Semele solida* is able to use the organic matter deposited on the surface sediments that organic matter would be an alternate energy source during autumn and winter. A similar explanation has been also given for the continuous

reproductive cycle of the intertidal bivalve *Diplodonta inconspicua* at the same site (Clasing et al. 1998).

The comparison between gametogenic cycles of subtidal versus intertidal populations shows that intertidal populations exhibit a slower development of the different stages of the reproductive cycle. Landers (1954) suggested that intertidal individuals of *Mercenaria mercenaria* living in the east coast of USA spawn earlier than subtidal individuals. Borrero (1987) also found that the beginning of gametogenesis and spawning in *Geukensia demissa* (Dillwyn) occurs earlier in low intertidal populations than high intertidal populations. Walker & Heffernan (1994) showed that the reproductive pattern of populations of *Mercenaria mercenaria* of Georgia (USA) is affected by the emersion time. On the other hand, Eversole et al. (1980) did not find differences in the reproductive parameters of subtidal and intertidal populations of the same species in the coast of South Carolina. Brousseau (1995) compared the results obtained for intertidal populations of *Crassostrea virginica* (Gmelin) in Long Island (USA) with those obtained before by Loosanoff (1942) 40 years earlier for subtidal populations, and concluded that not much difference exists as far as timing of the reproductive cycle is concerned.

The comparison of potential fecundity showed that the values estimated for *E. macha* were higher than those for *T. dombeii* and *M. edulis*. These differences may well be related to volume differences in gonadal tissue among species, a parameter directly correlated to the body size of individuals, i.e., the larger *E. macha* probably has larger gonads. Although no estimation of volume of gonads was carried out in this study, the body length of *E. macha* is longer than that of the other two species (see Figure 2). The potential fecundity of *E. macha* and *M. edulis* studied here is similar to other species of Chilean bivalves with plantotrophic larvae such as *Aulacomya ater* (Molina), *Choromytilus chorus* (Molina), *Venus antiqua* (King & Broderip), *Eurhomalea rufa* (Lamarck), *Mesodesma donacium* (Lamarck), and *Prothothaca thaca* (Molina) (Lozada, 1989). The results of this study showed that for *E. macha* and *T. dombeii* the potential fecundity was lower farther south. Differences in body size among sites may also be invoked to explain that result, i.e., the larger females collected farther north (Jaramillo et al., 1998, unpublished data) had larger gonads and thus, more potential fecundity.

However, the eventual effect of body size on size of gonads and potential fecundity seems to not affect mean sizes of oocytes. As a matter of fact, our results did not show any consistency. Thus, subtidal and intertidal populations of *T. dombeii* and *M. edulis* did not show significant differences in mean sizes of oocytes. This is different to the findings of other authors, such as Walker & Hefferman (1994) who found in England that subtidal populations of *Mercenaria mercenaria* have larger gonads and more oocytes than specimens living higher up

the coast. Also, Harvey & Vincent (1989, 1991) found that differences in exposure time to air during low tide is coincident with differences in potential fecundity of *Macoma balthica* inhabiting tidal flats of the Saint Lawrence Estuary (Canada).

No studies dealing with diameter of oocytes exists for *E. macha* and *M. edulis*. The mean diameter determined in this study for *T. dombeii* is smaller than that mentioned for other populations of the same species (Lozada, 1989). Interannual variability in mean size of oocytes may exist, as has been shown for *Macoma balthica* (L.) in Canada (Harvey et al., 1993) and *Mercenaria mercenaria* (L.) in South Carolina (USA) (Manzi et al., 1985).

In conclusion, there is variability in the timing of the gametogenic cycle of *E. macha*, *T. dombeii*, and *M. edulis* studied along the coast of southern Chile, i.e., the length of spawning and ripe stage periods varied. This is a key issue as far as management issues are concerned. Any management plan must take into account the geographic variability in the gametogenic cycles described here when closing seasons and minimum size of harvesting are determined. Due to the economic importance of many bivalves along the Chilean coast, there is an urgent need to evaluate the timing of gametogenic cycles of these species along their full geographic ranges.

Acknowledgments. We thank Iván Céspedes (Instituto de Fomento Pesquero, Talcahuano), Sandro Araneda (Valdivia), David Patiño (Universidad Austral de Chile, Maullín), Marco Lardies (Valdivia), and Nersio Saldivia (Instituto de Fomento Pesquero, Ancud) who collected the samples. This study was financed by Project FIP N° 96-46 "Estudio biológico pesquero de los recursos almeja, navajuela y huepo en las VIII y X Regiones." We are grateful to Orlando Garrido (Instituto de Embriología, Universidad Austral de Chile) who assisted us with the stereometric technique. MHA was funded by Dirección de Investigación y Desarrollo, Universidad Austral de Chile during the final data analyses and manuscript preparation. EJ acknowledges financial support from FONDAP O & BM (Programa Mayor N° 3) while preparing the manuscript.

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